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Full-length Article

Reciprocal relationship between membrane type 1 matrix metalloproteinase and the algesic peptides of myelin basic protein contributes to chronic neuropathic pain

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ABSTRACT

Myelin basic protein (MBP) is an auto-antigen able to induce intractable pain from innocuous mechanical stimulation (mechanical allodynia). The mechanisms provoking this algesic MBP activity remain obscure. Our present study demonstrates that membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) releases the algesic MBP peptides from the damaged myelin, which then reciprocally enhance the expression of MT1-MMP in nerve to sustain a state of allodynia. Specifically, MT1-MMP expression and activity in rat sciatic nerve gradually increased starting at day 3 after chronic constriction injury (CCI). Inhibition of the MT1-MMP activity by intraneural injection of the function-blocking human DX2400 monoclonal antibody at day 3 post-CCI reduced mechanical allodynia and neuropathological signs of Wallerian degeneration, including axon demyelination, degeneration, edema and formation of myelin ovoids. Consistent with its role in allodynia, the MT1-MMP proteolysis of MBP generated the MBP69-86-containing epitope sequences *in vitro*. In agreement, the DX2400 therapy reduced the release of the MBP69-86 epitope in CCI nerve. Finally, intraneural injection of the algesic MBP69-86 and control MBP2-18 peptides differentially induced MT1-MMP and MMP-2 expression in the nerve. With these data we offer a novel, self-sustaining mechanism of persistent allodynia via the positive feedback loop between MT1-MMP and the algesic MBP peptides. Accordingly, short-term inhibition of MT1-MMP activity presents a feasible pharmacological approach to intervene in this molecular circuit and the development of neuropathic pain.

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1. Introduction

Mechanosensation, a process of sensing and transducing mechanical stimuli into an electrochemical signal, depends on physical connectivity between extracellular matrix and cytoskeletal networks of the specialized cells. The mechanosensory networks, while ubiquitous in all living organisms, are especially complex in the human nervous system. In patients suffering from peripheral nervous system (PNS) damage due to trauma, cancer, diabetes, chemotherapy, viral pathogens and other causes, the network dysfunction may lead to a paradoxical state of mechanical allodynia (Treede et al., 2008). Mechanical allodynia (pain from non-painful mechanical stimuli) is a devastating state of pain associated with daily activities, such as wearing clothing or using bedding sheets, which often lasts long after incitement of PNS injury and remains refractory to current analgesics (Devor, 2009).

Mechanoselective A-afferent fibers of the PNS, responsible for non-painful touch and vibration sense, are electrically insulated by myelin. By enwrapping their cell membranes around axons, Schwann cells form a multi-lamellar myelin sheath with structurally, functionally and molecularly defined domains (internode, juxtaparanode and paranode). In myelin gaps (nodes of Ranvier),

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voltage-gated sodium channel clustering warrants rapid, saltatory conduction (Poliak and Peles, 2003). Demyelination of A-afferents contributes to the pathogenesis of mechanical pain hypersensitivity via disruption in this precise molecular and structural signature, including ectopic insertion of ion channels (Devor, 2009; Kobayashi et al., 2008; Henry et al., 2009). Among such myelin-related changes contributing to mechanical allodynia we proposed is liberation of myelin auto-antigens, such as myelin basic protein (MBP), leading to the formation of the algesic complexes on A-afferents (Shubayev et al., 2016; Liu et al., 2012; Ko et al., 2016).

MBP is an intrinsically unstructured cationic protein, which interacts with anionic lipids and cytoskeletal proteins, including actin and tubulin, to regulate myelin compaction and structural assembly of the axon-glia unit (Boggs, 2006). As a putative auto-antigen, MBP exhibits the cryptic T cell epitopes that play an important role in the immunopathogenesis of autoimmune demyelinating conditions in the PNS (Kadlubowski and Hughes, 1979). According to our recent findings, a physical PNS injury also liberates these hidden MBP epitopes (Kobayashi et al., 2008; Liu et al., 2012), injection of the synthetic peptides encoding these epitopes into an intact sciatic nerve is sufficient to produce a robust and long-lasting allodynia (Liu et al., 2012; Ko et al., 2016). While the chronic phase of MBP-induced allodynia relies on T cell activity, as shown using athymic nude rats (Liu et al., 2012), the molecular events initiating the release of the algesic MBP peptides in the course of painful PNS injury have yet to be elucidated.

The matrix metalloproteinase (MMP) family of Zn^{2+}-binding, Ca^{2+}-dependent endopeptidases conduct pericellular and extracellular proteolysis and, as a result, regulate the functionality of multiple contractile, adhesion and ion channel proteins and proteoglycans within the biomechanical cell networks (Lu et al., 2011; Mrkonjic et al., 2016). Evidence exists that, via the activation of the inflammatory cytokines (Myers et al., 2006; Dev et al., 2010; Shubayev and Myers, 2002; Kawasaki et al., 2008), ion channels (Remacle et al., 2015; Kim et al., 2012), and myelin sheath proteins, including MBP (Liu et al., 2012; Kadlubowski and Hughes, 1979; Bennett and Xie, 1988), MMP activity regulates the pronociceptive signaling associated with PNS injury. Several proteases among the fifteen MMP family members upregulated in the damaged PNS (Kim et al., 2012; Chernov et al., 2015) display a redundant ability to proteolyze MBP (Chandler et al., 1995; Gijbels et al., 1993; Proost et al., 1993; D'Souza and Moscarello, 2006; Shiryaev et al., 2009a,b). Based on these data, we proposed that a continuous release of the algesic MBP peptides in the damaged nerve relies on the catalytic activity of the several MMP family members (Kobayashi et al., 2008; Shubayev et al., 2016; Liu et al., 2012).

Pericellular activity of membrane type MT1-MMP/MMP-14 controls both the cell biomechanical properties (Mrkonjic et al., 2016; Strongin et al., 1995) and the level of the MBP proteolytic degradation (Shiryaev et al., 2009a,b). Herein, using a model of painful mononeuropathy, we provide the first evidence that MT1-MMP proteolysis causes the release of the algesic MBP peptides in the damaged PNS. The algesic MBP peptides enhance the expression of MT1-MMP and other MMPs in the nerve. This maladaptive feedback loop feeding self-sustaining mechanical pain hypersensitivity is efficiently interrupted using a short-term, selective MT1-MMP therapy.

2. Methods

2.1. Reagents

Routine reagents were purchased from Sigma. The function-blocking murine MT1-MMP monoclonal 9E8 antibody (mAb-9E8) Ingvarsen et al., 2013 was described earlier (Shiryaev et al., 2013; Ingvarsen et al., 2013); human MT1-MMP monoclonal DX2400 antibody (hAb-DX2400) was kindly provided by Kadmon (New York, NY) Devy et al., 2009. Human IgG1 was obtained from Abcam (ab184886). MBP69-86 (THYGSLPQKSQRTQDENPVV) and MBP2-18 (ASQKRPSQRSKYLATAS) peptides, derived from rat MBP sequence (GenBank, #CAA10804), protected N- and C-terminally by acetylation and amidation, respectively, were synthesized by GenScript. Human MBP (18.5 kDa isoform, GenBank #AAH08749) was purchased from Biodesign. The detection antibodies included mouse monoclonal (3G4/MAB1767) and rabbit polyclonal (AB8345) MT1-MMP antibody from EMD Millipore; mouse monoclonal MMP-2 antibody (MAB3308, Millipore); rabbit polyclonal S100B antibody (Z0311, Dako); rabbit polyclonal Iba1 antibody (019-19741, Wako); mouse monoclonal CD68 antibody (MCA341R, Serotec); and rabbit polyclonal antibody generated against the synthetic MBP peptide corresponding to amino acids 69–86 of the guinea pig protein (AB5864, Millipore).

2.2. Animal models and therapy

Sprague-Dawley rats (Envigo, 8–10-week-old, female) were housed in plastic cages in a temperature-controlled room with a 12-h light–dark cycle and free access to food and water. The procedure and testing were conducted during the light cycle. Under 4% isoflurane (Isothesia, Henry Schein) anesthesia, the common sciatic nerve was exposed unilaterally at the mid-thigh level through a gluteal muscle-splitting incision. Three loosely constrictive chromic gut ligatures were applied to produce the chronic constriction injury (CCI) model Bennett and Xie, 1988. At day 3 after CCI, hAb-DX2400, mAb-9E8 or control IgG1 (1.0 mg/ml, each), diluted in PBS (Steris Labs) vehicle, were administered by intraneural (i.n.) injection into the nerve fascicle (the CCI site) in a 10 μl volume using a 33-gauge needle. In a separate set of naïve rats, a single bolus i.n. injection of MBP peptide (50 μg in 5 μl PBS) was administered into the sciatic nerve fascicle. Animals were sacrificed by intraperitoneal injection of Euthasol (100–150 mg/ml; Virbac Animal Health). All animal procedures were performed according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the protocols approved by the Institutional Animal Care and Use Committee at the Veterans Affairs San Diego Healthcare System, and complied with ethical guidelines of the International Association for the Study of Pain.

2.3. Von Frey testing

Sensitivity to non-noxious mechanical stimuli was measured by von Frey testing using the up-and-down method (Chaplan et al., 1994) by an investigator unaware of the animal groups. Rats were acclimated to being on a suspended 6-mm wire grid for 5 days. The plantar surface of the hind paw within the sciatic nerve innervation area was stimulated using calibrated von Frey filaments (Stoelting). Baseline measurements were done for 3 consecutive days before CCI or i.n. MBP peptide injection, and then up to daily thereafter, as specified. Stimuli were applied for 2 s with a 0.4–15.0 g buckling force to the mid paw plantar surface with ascending filament stiffness until a paw withdrawal response occurred. Stimuli were separated by several-second intervals or until the animal was calm with both hind paws placed on the grid. The consecutive way of applying filaments was continued until six responses were recorded. The 50% threshold was calculated as described (Chaplan et al., 1994).

2.4. Neuropathology

Sciatic nerve segments were excised and post-fixed for 48 h at 4 °C using 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4.

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